EGG-VOLUME AND FERTILIZATION MEMBRANE.

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The question of fertilization and egg-volume seems to come up periodically for renewed research and discussion in spite of the papers already written on the topic.[1] In view of the disagreement as to the facts of volume changes at the moment of fertilization the writer wishes to submit the following evidence that was put in a paper in December, 1904, from observations made in that year on two species of echinoderms, and that hitherto has not been published. The work was done at the Timothy Hopkins Sea-side Laboratory of Stanford University, California.

The microscope images of the eggs were projected by a camera lucida, and outline drawings were made of their greatest diameters. Upon each sheet of drawings a stage micrometer scale was also projected and drawn. The magnification was uniform throughout. The diameters given in sections 1–4 inclusive, of this paper, are of these drawings, and should be divided by 110 in each case to get the approximate natural diameter.

1. *Echinarachnius eccentrica.*[2] Eggs made to develop parthenogenetically by treatment with hypertonic salt solution are known to shrink and then to swell again upon return to normal sea-water. Mature eggs of *Echinarachnius eccentrica* put in optimum hypertonic solution for 1½ hours were observed to shrink from a mean normal diameter of 13.27 to a mean diameter of 11.6 mm. After return to normal sea-water for 1 hour the eggs showed diameters ranging from 12.86 to 13.18 mm. By the time cleavage began, and the eggs were still in 1 and 2-cell stages, the diameters ranged between 14 and 16 mm.; when in stages varying from the 4-cell to the morula stage, the diameters ranged between 14.5 and 18 mm. These measurements refer to the egg-cytoplasm, no membranes appearing on eggs so treated.

[1] For review of the literature and discussion of the problem see Lillie, F. R., "Problems of Fertilization," Chicago, 1919, pp. 147–154; also Glaser, Otto: "Fertilization, Cortex and Volume," Biol. Bull., Vol. XLVII., pp. 274–283, 1924.

[2] Thanks are due to Professor Harold Heath, who kindly identified this species of sand-dollar for the writer.

Of the eggs fertilized with sperm those in 1 to 4-cell stages had diameters of egg-cytoplasm ranging from 13 to 15, and of fertilization membrane from 14.7 to 18 mm.; those in stages from 8-cells to blastulæ had diameters from 14.5 to 16 mm. for egg-cytoplasm, and 16.5 to 17 mm. for fertilization membrane. The number of eggs measured in each case was five only. But the extremes of a large number as well as mean sizes were taken in each case. The mean size of the hypertonic parthenogenetic eggs thus appear to reach nearly the same limit that the membrane does in eggs fertilized with sperm; the egg-cytoplasm of the former apparently takes up water as easily as the membrane of the latter. The writer's observations did not include a large enough number of these eggs at the moment of membrane formation to determine whether or not they showed a preliminary shrinking, as Otto Glaser[1] and others have observed in eggs of other species. One can only say that if the shrinking took place it must have occurred between the moment of the entrance of the spermatozoön and the moment just before the first cleavage.

2. *Asterina miniata* (*Brandt*) *Perrier*.[3]—When mature, and just before fertilization, a sample of seven eggs of this species measured 19, 19, 18.5, 19, 20, 19 and 20 mm., giving a mean of 19.25 mm. and an average of 19.21 mm. in diameter. When treated with suitable amounts of citric acid for a while and then returned to normal sea-water the eggs began to go into cleavage and develop into blastulæ.[4] A fertilization membrane could be seen reaching across the furrows between the cells of these eggs. They differed in this respect from the sand-dollar eggs that were made to go into cleavage by hypertonic solutions. But nevertheless the membranes did not free themselves from the outermost periphery of the egg-cytoplasm. The diameters, therefore, of egg-cytoplasm and fertilization membrane of *Asterina miniata* so treated underwent equal changes; they ranged between 19 and 21, giving a mean diameter of 20 mm. No measurements were made at the moment of treatment with the acid.

[3] Thanks are due to Dr. W. K. Fisher, who kindly identified this species of Asterina for the writer.

[4] The eggs of this species are known to be somewhat naturally parthenogenetic. In the series with the optimum concentration of the citric acid 79 per cent. swimming larvæ appeared at the end of 42 hours whereas in the control only 6 per cent. of the eggs were segmented and none were swimming. The average of all the series worked with showed a natural parthenogenesis to the extent of about 3.5 per cent.

When mature eggs of *Asterina miniata* are treated with sperm, the egg-cytoplasm during early stages of cleavage has an average diameter of 17.7 and the fertilization membrane one of 21 mm. This is shown in the table.

EGGS OF *Asterina Miniata* FERTILIZED WITH NORMAL SPERM.

Stage of Cleavage.	Diam. of Egg Cytoplasm.	Diam. of Fertilization Membrane.	Stage of Cleavage.	Diam. of Egg Cytoplasm.	Diam. of Fertilization Membrane.
1-cell.....	18	20	2-cell.......	18	20
1-cell.....	17	20.5	2-cell.......	18.5	22
1-cell.....	17	20	2-cell.......	18.7	21
1-cell.....	17	21.5	2-cell.......	17.5	22
1-cell.....	18	22.5	2-cell.......	18.5	22
1-cell.....	18	25	2-cell.......	18.7	20.5
4-cell.....	18	21	2-cell.......	19.2	22
2-cell.....	15	20	2-cell.......	19.0	20.5
2-cell.....	15	20	Average....	17.7	21.0

The mean diameters in these observations are 17.1 for the egg-cytoplasm and 22.5 for the membrane. The averages thus show less deviation than do the mean numbers. The amount of shrinking of the egg-cytoplasm comparing the mean diameters before and after fertilization is 11.1 per cent.; comparing the average diameters it is 7.8 per cent.

During the early stage of normal fertilization, then, the eggs of *Asterina miniata* may be said to show a marked shrinking of the egg-cytoplasm.

These figures are of the same order as Glaser (1914) observed for the reduction in diameters of just fertilized *Arbacia* eggs (from 1.4 to 14.5 per cent.) and *Asterias* eggs (from 10 to 17 per cent.); and Okkelberg [5] in volume reduction of eggs of the brook lamprey, 13.4 per cent. Just lately (1924) Glaser [1] repeated the measurements of Arbacia, using an improved method in order to prevent possible flattening of eggs before the membrane has been elevated (first suggested by Reighard, see Okkelberg, *loc. cit.*, p. 97, footnote 2), and finds that the percentage reduction of diameter is less than in his earlier work, but still a demonstrable mean of 3 per cent.

4. In spite of the meagerness of observations (the lack of measurements on the sand-dollar egg during the earliest stages following insemination, the lack of a more perfected treatment to

[5] Okkelberg, "Volumetric Changes in the Egg of the Brook Lamprey . . . after Fertilization," BIOL. BULL., Vol. XXVI., pp. 92–99, 1914.

induce parthenogenesis) the data still furnish one or two points of further interest.

The shrinking of the eggs of *Echinarachnius eccentrica* when subjected to optimum hypertonic solution for parthenogenetic development was from the mean diameter 13.27 before, to one of 11.6 mm. at the end of the treatment, or a reduction of 12.6 per cent. After return to normal sea-water, and by the time cleavage completed the 2-cell stage, the mean diameter was 15 mm.—an increase of 13 per cent.; by the time the eggs were in stages ranging from 4-cell to morulæ the mean diameter was 16.2 mm. showing a total increase of 22 per cent.

In the case of the inseminated eggs of this species the fertilization membrane (assuming it to be present on the unfertilized egg) showed an increase, while developing to the 4-cell stage, from a mean of 13.27 to one of 16.3 mm.—an increase of 22.8 per cent.; eggs in stages of 8-cells to blastulæ showed an increase to a mean diameter of 16.75 mm. or a mean total increase of 26.2 per cent.

In the case of *Asterina miniata* the increase in the average total diameter of fertilization membrane of inseminated eggs is from 19.21 to 21.0 scale divisions or one of 9.3 per cent, comparing averages the increase is 16.8 per cent.; of the acid treated parthenogenetic eggs the increase of both egg-cytoplasm and fertilization membrane (as in the case of hypertonic parthenogenetic eggs of the sand-dollar) is equal, and is the difference between 19.25 and 20.0 mm., or an increase of 3.9 per cent.

5. It may be of further interest to calculate the approximate mean actual diameters and actual volumes [6] of these eggs. If we assume them to have been spheres in all cases we have the following: The mature unfertilized egg of *Echinarachnius eccentrica* has a mean diameter of 120 μ, from which its volume must be about .0009 mm.^{3}; the optimum hypertonic shrinking gave a diameter of 105 μ, or a volume of .00061 mm.^{3}, representing a volume reduction of 33 per cent.

The mean actual diameter of mature *Asterina miniata* eggs is 174.6 μ, representing a volume of .00278 mm.^{3}; the mean diameter of egg-cytoplasm just after fertilization is about 161 μ,

[6] These volumes are calculated by multiplying the cube of the radius by 4.18 in each case. The radius is found by dividing the mean projected greatest diameter of the egg (that given in the text) by 2 x 110, 110 being the magnification.

from which the volume must be about .00217 mm.³, representing a volume reduction of 21.9 per cent. The fertilization membrane on the other hand presented a diameter of 191 μ and therefore enclosed a volume of .00364 mm.³; this represents an increase of volume capacity of 30.8 per cent.

The above observations are summed up in the following syllabus for the purpose of ready comparison. It will be observed that the ratio of diameter and volume changes is roughly as 1 : 2.8, a purely geometrical ratio.

Summary.

Percentage Dimensional Changes in Two Species of Echinoderm Eggs.

Reduction in egg-cytoplasm:

Of *Echinarachnius eccentrica*

By hypertonic salt action—in diameter, 12.6; in volume, 33.3.

By insemination (not observed).

Of *Asterina miniata*

By citric acid treatment (not observed).

By insemination—in diameter, 7.8; in volume, 22.

Swelling of fertilization membrane:

Of *Echinarachnius eccentrica*

Parthenogenetic (hypertonic salt action) [7]—in diameter, 22.8; in volume, about 62.

Normally inseminated—in diameter 26; in volume, about 70.

Of *Asterina miniata*

Parthenogenetic (citric acid treatment) [4]—in diameter 3.9; in volume, about 11.

Normally inseminated—in diameter 9.3; in volume, 31.

No special technical procedure was resorted to, to make certain that the eggs in these experiments were always spherical. The writer cannot, therefore, be quite certain that the diameters given above are those of perfect spheres and accordingly that the volumes given are the exact volumes of the eggs observed. If,

[7] The membrane is assumed to be present here and coextensive with the egg-cytoplasm.

as has been pointed out by others, the eggs at one time may be disks flattened vertically (Reighard, Chambers [8]) and at another time ellipsoids, or pear-shaped objects, suspended at one end of their long axes, and yet at another time perfect spheres, then there is an unrelated error among the observations that renders them worthless.

In the cases here studied there exists only the possibility of the eggs being flattened vertically during the period before fertilization and then changing into spheres after the fertilization membranes are raised from the egg-cortex. This, however, affects only the reduction observed in the inseminated *Asterina* eggs.

No one will doubt that the observed reduction in size of the sand-dollar eggs during the bath in hypertonic salt represents a real reduction in volume. If the effect of the osmosis is a gelation there may have been a hardening of the egg-cytoplasm, but this hardening of itself could not change the egg-mass from a spheroid to a sphere. If the effect of the osmosis is an increase in surface tension then such a change in form may well take place.

The reduction in diameter of the inseminated *Asterina* eggs during the first stage of cleavage is of the same order of magnitude as was observed in the sand-dollar eggs in their hypertonic bath. While some of this reduction may have been due to reshaping, there also can be no doubt that some of it was due to loss of material on the part of the egg-cytoplasm. This material, as others have maintained, may be colloidal in part, but this observation supports the view that considerable water is given off from the egg together with the colloid.

It is remarkable that the percentage of swelling of the egg treated to the hypertonic salt bath, after return to normal sea-water, and the percentage swelling of the fertilization membrane of the inseminated egg should both be of the same order of magnitude. This swelling in neither case can be due to any considerable extent to a reshaping of egg-substance. However evident it is that the egg-cytoplasm of this parthenogenetic egg has undergone a change in permeability different from the egg-cytoplasm of the normally inseminated egg, it nevertheless appears that the limit to extension in the one case is the same as it is in the other. For since the beautiful demonstration of Chambers [8] there can be no doubt that the fertilization membrane is a preëxistant structure. One may take the limit in the swelling, therefore, to be the degree of permeability and elasticity of the membranes in both cases.

What has just been said of the eggs subjected to hypertonic solution appears also to be true of the eggs subjected to citric acid. For in this latter case the egg-cytoplasm also swells coextensively with the egg-membrane. It will be remembered that in these eggs the membrane could be seen bridging the furrows between the cells after cleavage, in this respect differing from the hyaline membrane that is observed to dip down and follow the furrow closely.[8] The limit to swelling in the artificially parthenogenetic Asterina egg thus also lies in the egg (fertilization) membrane. Only, the citric acid treatment seems to render the membrane somewhat less permeable to water or less elastic, or both less permeable and less elastic, than does the treatment with normal sperm.

In conclusion it may be added that while the above observations contribute little or nothing of a decisive character to the problem, they nevertheless do add to the attractiveness of the space-time method. With the newer technique a thoroughly systematic application ought to yield results not only decisive but also important.

The Johns Hopkins University,
December 18, 1924.

[8] Chambers, Biol. Bull., Vol. XLI., pp. 318–350, 1921.